DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on April 5, 2011 has been entered.

Claims 1-3, 5, and 8-9 are currently pending.

Claim 1 has been amended.

Withdrawn Rejections

2. The rejections made under 35 USC 103(a) in section 6 of the Office Action of November 5, 2010 are withdrawn in view of the amendments made to the claims and the Applicants arguments.

Claim Objections

3. Claim 1 is objected to for having a typographical error. Specifically the word "and" in claim 1 step (i) should be deleted.

Claim Rejections - 35 USC § 112 1st paragraph

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3, 5, and 8-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "healthy" in claim 1 is a relative term which renders the claims indefinite. The term "healthy" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. This rejection could be overcome by amending the claims to recite i.e., “an individual without cancer”.

Claim Rejections - 35 USC § 112 1st paragraph

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3, 5, and 8-9 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Nature of the Invention

The claims are drawn to an assay for assessing the risk of cancer in a healthy individual. The method comprises (i) isolating a population of cells from normal tissue

of said individual, (ii) quantitatively determining the frequency of epimutation present in a tumor suppressor gene in said population of cells, and (iii) assessing said risk based on said frequency of epimutation present in a tumor suppressor. The wherein clauses state that (A) the epimutation of said tumor suppressor gene is associated with said cancer, (B) the epimutation is DNA methylation, (C) said tumor suppressor gene is other than one that is subject to normal parent of origin-specific expression, and (D) wherein said cancer is selected from the group consisting of breast cancer, ovarian cancer, malignant melanoma, pancreatic cancer, prostate cancer, retinoblastoma, leukemia, lymphoma, renal cancer, endometrial cancer, paraganglioma, phaeochromocytoma, basal cell carcinoma, soft tissue carcinoma, brain tumors, testicular cancers, and gynecological malignancies. The nature of the invention requires a reliable association between the presence of a methylated gene in a tissue sample obtained from a healthy patient and the risk of various types of cancer.

Scope of the Claims:

The claims are drawn to an assay for assessing the risk of cancer in a healthy individual. The fourth wherein clauses states that the cancer is selected from the group consisting of breast cancer, ovarian cancer, malignant melanoma, pancreatic cancer, prostate cancer, retinoblastoma, leukemia, lymphoma, renal cancer, endometrial cancer, paraganglioma, phaeochromocytoma, basal cell carcinoma, soft tissue carcinoma, brain tumors, testicular cancers, and gynecological malignancies. In view of the recitation of phrase "healthy individual" the claims broadly encompass human and

non-human individuals. Further neither the claims nor the specification set forth what it means to be healthy.

The method comprises a first step of isolating a population of cells from normal tissue of the individual. The specification (page 9) states that the term "normal tissue" refers to any tissue which is substantially healthy and not showing any significant symptoms or signs of disease (e.g. the tissue is not cancerous) and includes all normal somatic tissues. As such the claims broadly encompass any type of tissue sample i.e., breast tissue, liver tissue, skin, heart tissue, brain tissue etc.).

The method comprises a second step of quantitatively determining the frequency of epimutation present in a tumor suppressor gene in said population of cells. The first wherein clause states that the epimutation of said tumor suppressor gene is associated with said cancer. The second wherein clause states that the epimutation is DNA methylation. The third wherein clause states that the tumor suppressor gene is other than one that is subject to normal parent or origin specific expression. The claims encompass a large genus of tumor suppressors.

The method comprises a third step of assessing the risk based on said frequency of epimutation present in a tumor suppressor. However the claim does not set forth how to assess the risk based on the frequency of epimutation present in a tumor suppressor. For example the claims do not set forth what frequency of epimutation is required to determine that the individual has an increased risk or decreased risk of cancer. Further the claims do not state what the risk is in comparison to.

Claim 2 is drawn to an assay wherein the normal tissue is either a normal hair follicle tissue or a normal tissue from the buccal cavity.

Claim 3 is drawn to an assay wherein the normal tissue is normal peripheral blood.

Claim 5 is drawn to an assay wherein the epimutation is present in the promoter or other regulatory region of the gene and is associated with transcriptional silencing of said gene.

Claim 8 is drawn to an assay wherein the epimutation is present in a gene selected from the group consisting of *hMLH1, hMSH2, APCIA, APCIB* and *p16.*

Claim 9 is drawn to an assay wherein the epimutation is present in *hMLHI.*

Teachings in the Specification and Examples:

The specification (pages 14-15) discloses that peripheral blood was collected from healthy donors. DNA was extracted and then subjected to methylation screening. The specification discloses that 12 out of 22 healthy donors had some detectable level of hyper methylated hMLH1. The specification discloses that 18 out of 29 healthy donors had some detectable level of hyper methylated p16. The specification (page 15) states that these results indicate that healthy individuals commonly carry a detectable level of cells in which the hMLH1 or p16 gene is epimutated. The specification states that inactivation of one allele of either hMLH1 or p16, and indeed many other tumor suppressor genes, is known to predispose a cell to become malignant through loss or inactivation of the second allele. As such these individuals are considered as being at

risk of becoming malignant through loss or inactivation of the second allele, and this risk is higher than that of cells that do not carry the epimutation.

The Predictability or Unpredictability of the Art:

While the state of the art with regard to the detection of methylation is high, the unpredictability with regard to associating methylation levels in normal tissue obtained from a healthy individual with the risk of cancer is even higher. The unpredictability is discussed below.

In the instant case it is highly unpredictable if it is possible to assess the risk of cancer in a healthy individual by assaying a population of cells from normal tissue of the individual to determine the frequency of hMLH1 methylation present. The specification discloses that 12 out of 22 healthy blood donors had some detectable level of hyper methylated hMLH1. However there is no data in the specification which indicates how many of the 12 healthy blood donors with hyper methylated hMLH1 eventually developed cancer. Additionally there is no data in the specification which indicates how many of the 10 healthy blood donors without hyper methylated hMLH1 remained cancer free. Without this information it is impossible to determine if healthy individuals with hyper methylated hMLH1 actually have a higher risk of developing cancer in comparison to healthy individuals without hyper methylated hMLH1. Even if there was data which supported the hypothesis that healthy blood donors with hyper methylated hMLH1 will develop cancer, the specification does not set forth what level of frequency

of methylation is required to determine that the individual has an increased risk of cancer or that the individual has a decreased risk of cancer.

In the instant case it is highly unpredictable if it is possible to assess the risk of cancer in a healthy individual by assaying a population of cells from normal tissue of the individual to determine the frequency of methylation of p16. The specification discloses that 18 out of 29 healthy blood donors had some detectable level of hyper methylated p16. However there is no data in the specification which indicates how many of the 18 healthy blood donors with hyper methylated p16 eventually developed cancer. Additionally there is no data in the specification which indicates how many of the 11 healthy blood donors without hyper methylated p16 remained cancer free. Without this information it is impossible to determine if healthy individuals with hyper methylated p16 actually have a higher risk of developing cancer in comparison to healthy individuals without hyper methylated p16. Even if there was data which supported the hypothesis that healthy blood donors with hyper methylated p16 will develop cancer, the specification does not set forth what level of frequency of methylation is required to determine that the individual has an increased risk of cancer or that the individual has a decreased risk of cancer.

In the instant case it is highly unpredictable if it is possible to assess the risk of cancer in a healthy individual by assaying a population of cells from normal tissue of the individual to determine the frequency of methylation of hMSH2, APC1A, or APC1B. In the instant case the specification does not teach assaying the methylation frequency of

these genes in samples derived from healthy blood donors. Therefore it hasn't been established that hyper methylation of these genes can even be detected in normal tissue from healthy individuals. In the absence of evidence to the contrary it is highly unpredictable if these genes can be used to assess the risk of cancer.

While the specification exemplifies detecting hyper methylation of hMLH1 and p16 in normal peripheral blood samples obtained from a healthy individual, it is highly unpredictable if it is possible to detect hyper methylation of hMLH1 and p16 in any other types of normal tissue samples (i.e., breast tissue, liver tissue, skin, heart tissue, brain tissue, hair follicle tissue, and buccal tissue). For example the specification (page 14) teaches that CpG methylation of hMLH1 was detected in normal colon tissue from an individual with colorectal cancer however, methylation of hMLH1 could not be detected in the patient's peripheral blood, hair, and buccal mucosa. Additionally it is noted that Michels (Experimental Gerontology 2010 Vol 45 pages 297-301) teaches that DNA methylation is tissue specific and may be cell type specific and a certain DNA methylation pattern found in one specific tissue does not permit inferences about its variation across different tissues and possibly not even across different cell types in the same tissue (page 299, col 1). As such assaying a population of cells from any normal tissue to assess the risk of cancer in a healthy individual is a highly unpredictable endeavor.

Because the claims encompass a method wherein the tumor suppressor is associated with the cancer, it is relevant to point out that the specification does not teach which tumor suppressors are associated with each of the cancers encompassed

by the claims. Duffy (European Journal of Cancer 2009 pages 335-346) teaches that just because a gene is methylated in one cancer it does not mean that the gene will be methylated in every type of cancer. For example the hMLH1 gene is methylated in colorectal and gastric cancers but is rarely methylated in esophageal cancer and hepatoma. BRCA1 is methylated in breast and ovarian cancers but not in many other types of cancer. Methylation of p73 and p15 genes appears to be present almost exclusively in hematological malignancies (page 337, col 1). As such it is highly unpredictable as to which tumor suppressor (*hMLH1, hMSH2, APCIA, APCIB* and *p16)* should be assayed when trying to assess the risk of breast cancer, ovarian cancer, malignant melanoma, pancreatic cancer, prostate cancer, retinoblastoma, leukemia, lymphoma, renal cancer, endometrial cancer, paraganglioma, phaeochromocytoma, basal cell carcinoma, soft tissue carcinoma, brain tumors, testicular cancers, and gynecological malignancies.

In the instant case it is unpredictable as to whether the results obtained in human individuals could be extrapolated to non-human individuals. Knowledge that a particular tumor suppressor is hyper methylated in normal tissue of one organism (i.e. humans) does not allow one to conclude that the tumor suppressor will also be hyper methylated in normal tissue with other organisms and be associated with an increased risk of cancer. This finding of unpredictability is supported by Feng (PNAS 2010 Vol 107 No 19 pages 8689-8694). Specifically Feng teaches that although DNA methylation likely has a conserved role in gene silencing, the levels and patterns of DNA methylation appear to vary drastically among different organisms (abstract). As such it is unpredictable as to

whether the claimed method can be used to assess the risk of cancer in healthy non-human individuals.

Additionally it is noted that the art recognizes that there are several challenges associated with using epigenetic biomarkers. For example Michels (Experimental Gerontology 2010 Vol 45 pages 297-301) teaches that the sample size has important implications for the results of an epigenetic study. Michels teaches that a sufficiently large sample size is a fundamental requirement of a high quality study in epigenetics (page 299, col 1). This is relevant to the instant claims because the sample sizes were quite small- only 22 healthy blood donors in the hMLH1 study and only 29 healthy blood donors in the p16 study. Additionally Michels teaches that a study of the influence of a specific DNA methylation pattern on a disease outcome could be confounded by a variety of variables that affect methylation and are also risk factors for the disease. For example age is a likely confounder of a study in epigenetics since the DNA methylation profile changes with age and age increases the risk for most diseases (page 299, col 2).

Further Duffy (European Journal of Cancer 2009 pages 335-346) teaches that there are several unresolved issues in the use of methylated genes as cancer markers. Duffy teaches that the use of methylated genes for risk identification or aiding cancer diagnosis is based on the assumption that methylation at specific sites in the promoter regions of certain genes is confined to malignancy or premalignant lesions. Other factors, however, may affect gene methylation such as aging and benign diseases. Duffy refers to a study that examined the effect of aging on DNA methylation in non-malignant human prostate tissue. A significant increase in DNA promoter methylation

with age was found for several genes including *RARß2*, *RASSF1A* and *GSTP1*. The implication of this finding is that studies investigating a potential diagnostic utility for methylated genes should as a minimum include age-matched controls. Duffy also teaches that certain benign diseases, especially benign tumors, may also exhibit altered gene methylation. Little or no research has been carried out in this area but is essential prior to gene methylation assays being recommended for cancer screening and/or diagnostic purposes (page 341, col 1).

Finally the specification (page 15) asserts that these results indicate that healthy individuals commonly carry a detectable level of cells in which the hMLH1 or p16 gene is epimutated. The specification states that inactivation of one allele of either hMLH1 or p16, and indeed many other tumor suppressor genes, is known to predispose a cell to become malignant through loss or inactivation of the second allele. As such these individuals are considered as being at risk of becoming malignant through loss or inactivation of the second allele, and this risk is higher than that of cells that do not carry the epimutation. However the specification has not provided any statistical analysis of this risk. Therefore it's unclear how much of a risk these individuals have in comparison to individuals that do not carry the epimutation and whether this risk would be considered statistically significant. The prior art of Thisted (The University of Chicago1998) provides guidance as to what is required to indicate that an association is statistically significant (Thisted teaches that it has become scientific convention to say that a P-value of 0.05 is considered significant (p.5 - What does it mean to be 'statistically significant'), and that values above the conventional reference point of 0.05

would not be considered strong enough for the basis of a conclusion). Therefore without p- values (or an equivalent measure), one cannot determine if the results of a particular study are statistically significant.

Quantity of Experimentation:

Due to the highly unpredictable nature of the invention, a large amount of additional experimentation is required. For example, such experimentation may involve a large prospective clinical study in which a representative number of different normal tissue samples (i.e., blood, buccal tissue, hair) is collected from healthy individuals and methylation of a panel of tumor suppressor genes (i.e., hMLH1, hMSH2, APC1A, APC1B, and p16) is assayed. Then each individual would have to be followed over time to determine if they ever developed breast cancer, ovarian cancer, malignant melanoma, pancreatic cancer, prostate cancer, retinoblastoma, leukemia, lymphoma, renal cancer, endometrial cancer, paraganglioma, phaeochromocytoma, basal cell carcinoma, soft tissue carcinoma, brain tumors, testicular cancers, and gynecological malignancies. Then statistical analysis would have to be performed to determine if healthy individuals that had methylation in their normal tissue had a significantly higher risk of developing cancer than those healthy individuals who did not have methylation in their normal tissues. The results would then have to be validated. In addition to clinical validation, assays for methylated genes must be robust, simple, standardized, evaluated in external quality assurance schemes and made available at affordable costs. Only then, can patients expect to benefit from measurement of these markers. Even if such a large amount of experimentation were to be performed, the results are

highly unpredictable. This would require years of inventive effort with no guarantee of success. The specification has provided only an invitation to experiment.

Conclusions:

Taking into consideration the factors outlined above, including the nature of the invention and breadth of the claims, the state of the art, the level of skill in the art and its high level of unpredictability, the guidance provided by the applicant and the specific examples, it is the conclusion that an undue amount of experimentation would be required to make and use the invention as broadly claimed.

Conclusion

6. No Claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Amanda M. Shaw whose telephone number is (571) 272-8668. The examiner can normally be reached on Mon-Fri 7:30 TO 4:30. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached at 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Amanda Shaw/
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